IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Robert J. HARIRI	Confirmation No.:	1042
Serial No.:	10/074,976	Art Unit:	1632
Filed:	February 13, 2002	Examiner:	Li, Qian Janice
For:	TISSUE MATRICES COMPRISING PLACENTAL STEM CELLS, AND METHODS OF MAKING THE SAME [1]	Attorney Docket No.: (CAM)	9516-101-999 (501872-999100)

DECLARATION OF QIAN YE, PH.D. UNDER 37 C.F.R. § 1.132

I, Qian Ye, Ph.D., declare the following:

1. I have been employed for more than five years by Anthrogenesis Corporation (now part of Celgene Corporation) as a Senior Scientist. It is my understanding that Anthrogenesis Corporation is the assignee of record the above-captioned patent application.

2. I earned a Bachelor of Science degree in Microbiology at Xiamen University (China) in 1986. In 1990, I earned a Master of Science degree in Botany, also at Xiamen University (China). I earned a Doctor of Philosophy degree in Biomedical Science at the Mount Sinai Medical Center, The City University of New York in 1996. In addition, I have received postdoctoral training at Colombia University (1996-1997) and Memorial Sloan Kettering-Cancer Center (1997-2001). I have attached a copy of my Curriculum Vitae ("CV") to this Declaration as Exhibit A.

3. I have extensive experience in the area of stem cell technology. As the attached CV indicates, my expertise includes extensive experience in the design, execution and analysis of experiments relating to the production and proliferation of stem cells, including placental stem cells, as well as in the evaluation of such stem cells. I have published no fewer than five (5) abstracts related to stem cells.

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[1] As amended in the accompanying Amendment.

4. In addition, in my capacity as Senior Scientist at Anthrogenesis Corporation, I have executed and/or supervised experiments related to stem cells. Specifically, for example, I have designed and implemented research programs on the isolation, establishment, and the differentiation of stem cells from the human placenta.

5. I have read, and I am familiar with, Application No. 10/074,976 ("the '976 application"), filed February 13, 2002. It is my understanding that an Office Action, mailed January 19, 2006, was issued in connection with the '976 application. I have reviewed the claims that will be pending in the '976 application upon filing and entry of the response to the Office Action.

6. As described in the following paragraphs, and in the Figures included in Exhibit B, experiments performed by me, or familiar to me, demonstrate that the teaching provided in the specification of the '976 application allows for routine practice of the methods and compositions being claimed in the '976 application. In particular, the '976 application teaches methods that can routinely be practiced to yield the cells recited in the claims that will be pending in the '976 application.

7. The experiments presented in Exhibit B characterize populations of cells collected from human placentas that have been drained of cord blood and flushed to remove residual blood. The results of these experiments demonstrate that such populations comprise $CD34^-$ placental stem cells, including $CD34^-$ stem cells that are positive for OCT-4 or negative for SSEA3 or SSEA4; or $CD34^-$ stem cells that are positive for CD10, CD29, CD44, CD54, CD90, SH2, SH3 or SH4, and/or are negative for CD45.

8. The experiments discussed herein were performed according to the teaching presented in the '976 application. *See*, for example, pp. 15-28, specifically p. 16, line 7 to page 21, line 4; page 21, line 22 to page 24, line 8; page 26, line 2 to page 27, line 27; Examples 1 and 2, and FIGS. 1-3, which describe, *e.g.*, methods of collection of populations of cells comprising stem cells from human placentas that have been drained of cord blood and flushed to remove residual blood.

9. The methods described in paragraph 8, above, yield populations of cells comprising placental stem cells. As evidence that such populations comprise placental stem cells,

the populations comprise cells that can be differentiated into several different cell types (adipogenic, chondrogenic, or neural).

10. For example, Figure 1 shows that CD34$^-$ placental cells collected *via* the teachings as discussed in paragraph 8 can differentiate into an adipogenic lineage. In particular, CD34$^-$ placental cells cultured under adipogenic induction conditions for 28 days form vacuoles in their cytoplasm, as evidenced by Oil Red O dye, a dye specific to oil droplets in fat cells (Figure 1, top right photograph). RT-PCR was also used to assess the expression of adipocyte specific genes during adipogenic induction. The adipocyte specific genes Fatty Acid Binding Protein (FABP) and Peroxisome Proliferator Activated Receptor-gamma were found to be expressed starting at day 2 after induction (Figure 1, bottom). The expression of these genes was maintained throughout the entire 28-day induction period. These morphological and molecular characteristics are indicative of cells that have differentiated to an adipogenic lineage.

11. The CD34$^-$ placental cells collected *via* the teachings as discussed in paragraph 8 can also differentiate into cells of a chondrogenic lineage. In particular, Figure 2 shows that CD34$^-$ placental cells that were cultured under chondrogenic induction conditions for 28 days display a highly dense cartilage-like tissue, as evidenced by Periodic Acid Schiff ("PAS") staining, a histochemical reagent used to determine the presence of glycosaminoglycan produced by cartilage tissue (Figure 2, top middle and right photographs). During the induction course, RT-PCR analysis was also used to assess Collagen-II gene expression. The expression of Collagen II mRNA becomes apparent at approximately 2 weeks after induction and is sustained until day 28 (Figure 2, bottom). These morphological and molecular characteristics are indicative of cells that have differentiated to a chondrogenic lineage.

12. The CD34$^-$ placental cells collected *via* the teachings as discussed in paragraph 8 can also differentiate into cells of a neural, or neurogenic, lineage. In particular, as shown in Figure 3, CD34$^-$ placental cells that were cultured under neural induction conditions with 3-isobutyl-1-methylxanthine (IBMX) developed a neuron-like morphology. Moreover, the cells stained positive for glial fibrillary acidic protein (GFAP), a marker of glial cells and astrocytes, and neurofilament (NF), a marker of

differentiated neurons. These molecular characteristics are indicative of cells that have differentiated to a neural lineage.

13. Thus, because the $CD34^-$ placental cells can differentiate into cells of at least three lineages, they are stem cells. Moreover, it is noted that these results demonstrate that the placental stem cells, collected in the populations of placental cells, comprise stem cells that are different from hematopoietic stem cells, which differentiate solely into blood cell lineages. Indeed, the $CD34^-$ placental stem cells are non-hematopoietic.

14. Figures 4-6, described in more detail in paragraphs 15-17, below, depict fluorescence activated cell sorting (FACS) and RT-PCR data as referenced in paragraphs 18-20, below. These data show that populations of cells collected by the methods described in paragraph 8, above, comprise $CD34^-$ placental stem cells (paragraph 18). The data further show that the $CD34^-$ placental stem cells comprise cells that are positive for OCT-4 or negative for SSEA3 or SSEA4 (paragraph 19); or are positive for CD10, CD29, CD44, CD54, CD90, SH2, SH3 and/or SH4, and/or negative for CD45 (paragraph 20). The markers discussed herein were well known to persons of skill in the art as of the filing date of the '976 application, as were methods and compositions for detecting such markers. Such persons at that time would have known how to identify such markers and to use such markers for the characterization of cells, including stem cells.

15. Figure 4 is a table summarizing the results of FACS characterizations of stem cells present in populations of cells collected *via* the teachings discussed in paragraph 8, above. Cells from different perfusion experiments were given unique designations (*e.g.*, PLSC-1, PLSC-2, *etc.*).

16. Figure 5 shows an agarose gel of the results of RT-PCR, using OCT-4 specific primers, of mRNA from $CD34^-$ placental stem cells from perfusion experiments designated PLSC-27, PLSC-36, PLSC-44, PLSC-53 and PLSC-75. OCT-4 cDNA appears as a band on the gel. The lanes marked "–" are negative controls.

17. Figure 6 is another table summarizing the results of FACS characterizations of stem cells present in populations of cells collected in two experiments, designated YW2073 and YW2059, according to the teachings as discussed in paragraph 8, above.

18. The placental stem cells present in the populations of cells collected by the methods described in paragraph 8, above, comprise $CD34^-$ stem cells. *See, e.g.*, Figure 4, PLSC-1 to PLSC-3, PLSC-5 to PLSC-10, PLSC-15 to PLSC-21, PLSC-23, PLSC-26 and PLSC-27;[2] and Figure 5.

19. The placental stem cells, present in the population of cells collected by the methods described in paragraph 8, above, comprise $CD34^-$ stem cells that are positive for OCT-4 or negative for SSEA3, or SSEA4. *See* data in Figure 4 (PLSC-3 and PLSC-6 to PLSC-10) and Figure 5, which demonstrate that $CD34^-$ cells are present that are $OCT\text{-}4^+$; and Figure 6, which demonstrates that $CD34^-$ placental stem cells that are $SSEA3^-$ and $SSEA4^-$ are present. It is noted that the fact that such human placental stem cells are negative for SSEA3 and SSEA4 distinguishes these cells from human embryonic stem cells, which are positive for SSEA3 and SSEA4.

20. The $CD34^-$ placental stem cells present in the population of cells collected by the methods described in paragraph 8, above, comprise stem cells that are positive for CD10, CD29, CD44, CD54, CD90, SH2, SH3 and/or SH4, and/or negative for CD45. *See* data in Figure 4, PLSC-1 to PLSC-3, PLSC-5 to PLSC-10, PLSC-15 to PLSC-21, PLSC-23, PLSC-26, and PLSC-27. Cells were present that are $CD45^-$, $CD10^+$, $CD29^+$, $CD54^+$, $SH2^+$, $SH3^+$ and $SH4^+$ and are consistently also $CD44^+$ and $CD90^+$ (*see* Figure 4, PLSC-15 to PLSC-21; PLSC-23, PLSC-26 and PLSC-27) and $OCT\text{-}4^+$ (*see* Figure 4, PLSC-3 and PLSC-6 to PLSC-10).

21. The $CD34^-$ placental stem cells present in the population of cells collected by the methods described in paragraph 8, above, would not be expected to differentiate upon seeding onto a tissue matrix, without an additional inducement to differentiate.

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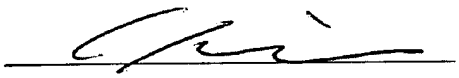
[2] Cells not indicated as being positive or negative for a particular marker were not tested for that marker.

22. In addition to being different from hematopoietic and embryonic stem cells, as explained above in paragraphs 12 and 18, respectively, the $CD34^-$ placental stem cells are also distinct from $CD34^-$ mesenchymal stem cells and cord blood-derived stem cells. For example, the $CD34^-$ human placental stem cells, collected according to the teachings as discussed in paragraph 8, above, can develop embryoid-like bodies in cell culture (*see* Figure 7), whereas mesenchymal stem cells and the cord blood-derived stem cells, whether $CD34^+$ or $CD34^-$, do not. Embryoid-like bodies are clusters of cells that the $CD34^-$ human placental stem cells tend to form after several days in culture under proliferating conditions. The clusters of cells are called "embryoid-like" because they morphologically resemble embryoid bodies produced by cultured embryonic stem cells, but are produced by $CD34^-$ placental stem cells, which are non-embryonic.

23. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and such willful false statements may jeopardize the validity of the application or any patents issuing thereon.



Qian Ye, Ph.D.

8/3/2006

Date

Qian Ye Ph.D.

EDUCATION:

1991-1996	Ph.D., Biomedical Science, Mount Sinai Medical Center, The City University of New York
1986-1990	M.S., Major in Botany, Xiamen University, China
1982-1986	B.S., Major in Microbiology, Xiamen University, China

POSTDOCTORAL TRAINING:

1997-2001	Cell Biology Program, Memorial Sloan Kettering-Cancer Center
1996-1997	Departments of Medicine and Pathology Columbia University College of Physicians and Surgeons

EXPERTISE:

Developmental Hematopoiesis

-Extensive experience in experimental design, data collection and analysis of proliferation and differentiation of hematopoietic stem cells from human and mouse using in vitro and in vivo systems

-Assays in evaluating hematopoietic stem cells including isolation of hematopoietic stem cells, colony forming cell assays, cobble-stone area assays (CAFCs), long-term bone marrow culture, long-term initiating cell assays (LTCICs), CFU-S

Animal Experiences

-Use of retrovirus system to express target genes in hematopoietic stem cells and analysis their effects on hematopoiesis in mouse by xenoengraftment

- Use of mouse model to study hematopoiesis and immunology

Flow-Cytometry

-Hands on experience of Becton-Dickson to perform cell cycle analysis, apoptosis, two-color and multiple-color analysis on protein expression

Molecular Biology and Biochemistry

-Extensive experiences in cDNA, genomic library screening

-Gene function analysis using retrovirus expression system

-Yeast two-hybrid system in analysis of protein-protein interactions

-Major DNA techniques including recombinant DNA cloning, Southern blot, Northern blot, PCR, RT-PCR, luciferase system in analysis of transcription regulation

-Major protein techniques including expression and purification protein from bacteria and mammalian cells (small or large scale), SDS-PAGE, Western-blot chromatography, immunoprecipitation, ELISA

Cell Biology

-Mammalian cell culture including cell lines, primary cells and ES cells, establishment of cell lines, cellular fractionation, conventional and confocal immunofluorescence microscope

AWARDS AND HONORS:

1999-2001	PHS Fellowship Award, National Heart, Lung, and Blood Institute, NIH
1996-1997	American Liver Foundation Postdoctoral Research Fellowship
1995-1996	American Liver Foundation Student Research Award
1995	American Society for Cell Biology Annual Meeting Travel Award
1994-1995	American Liver Foundation Student Research Award

RESEARCH AND WORKING EXPERICENCE:

Senior Scientist (5/2001-present)

Celgene, Cellular Therapeutics Division (Anthrogenesis Corp)

Responsibilities:

a. Design and implement research programs on the isolation, establishment, and multiple lineage differentiation stem cells from human placental and cord blood
b. Research programs for cell therapy pre-clinical studies
c. Assay development to study effects of small molecule drugs on the proliferation and cytokine directed differentiation of hematopoietic stem cells.
d. Assays development to study the effects of small molecules drugs on angiogenesis

Research Fellow (5/1997-5/2001)

Laboratory of Developmental Hematopoiesis, Memorial Sloan-Kettering Institute, New York
Mentor: Dr. Malcolm A.S. Moore

Project: Notch signaling on the regulation of hematopoietic stem cells

Research activities:

a. Cloning of cDNA for human Notch ligand Delta-like-1 (hDll1)
b. Expression and purification of Notch binding domain, the (DSL) domain, of hDll1 as a recombinant protein and studied its roles in regulating the proliferation and differentiation of hematopoietic stem cells.
c. Using retroviral vectors to express hDll1 and Notch receptors in mouse/human hematopoiteic stem/progenitor cells to analyze their roles in regulating hematopoiesis in vitro and in vivo using NOD/SCID mouse

Postdoctoral Fellow (4/1996-5/1997)

Department of Medicine and Cell Biology, Columbia University, New York
Mentor: Howard J. Worman, M.D.

Research Activities:

Functional analysis of domain-specific interactions between heterochromatin protein family and inner nuclear inner membrane protein lamin B receptor (LBR)

Graduate Research Assistant (9/1991-4/1996)

Department of Molecular Biology, Mount Sinai Medical Center, New York.
Mentor: Howard J. Worman, M.D.

Ph.D thesis: Protein-protein and protein-chromatin interactions at the nuclear envelope.

a. cDNA library screening, cloning of cDNA for human LBR, a nuclear membrane protein
b. Analysis of the interaction between LBR with DNA, chromatin and nuclear lamins
c. Analysis of interactions between major lamin proteins by yeast two-hybrid system
d. Identification of interactions between LBR and HP1 proteins with yeast two-hybrid screening

PUBLICATIONS:

1. **Ye, Q.,** Shieh, J-H, Morrone, G. and Moore, M.A.S. (2004) Expression of constitutively active Notch4 (Int-3) modulate myeloid proliferation and differentiation and promote expansion of hematopoietic progenitors. Leukemia. 2004 18:777-87.
2. Han, W., **Ye, Q.**, and Moore, MAS. (2000) A soluble form of human Delta-like-1 inhibits differentiation of hematopoietic progenitor cells. Blood 95:1616-1625.
3. **Ye, Q.**, Callebaut, I., Pezhman, A., Courvalin, J.C. and Worman, HJ. (1997). Domain-specific interactions of human HP1-type chromatin proteins and inner nuclear membrane protein LBR. J. Biol. Chem. 272:14983-14989.
4. **Ye, Q.**, and Worman, HJ. (1996). Interaction between an integral protein of the nuclear envelope inner membrane and human chromodomain proteins homologous to Drosophila HP1. J. Biol. Chem. 271:14653-14656.
5. Lin, F., Noyer, CM, **Ye, Q.**, Courvalin, J.C., and Worman, HJ. (1996). Autoantibodies from patients with primary biliary cirrhosis recognize a region within the nucleoplasmic domain of inner nuclear membrane protein LBR. Hepatology. 23:57-61.
6. **Ye, Q.**, and Worman, HJ. Protein-protein interactions between human nuclear lamins expressed in yeast. (1995) Exp. Cell Res. 219:292-298.
7. **Ye, Q.** and Worman. HJ. (1994). Primary structure analysis and lamin B and DNA binding of human LBR, an integral protein of the nuclear envelope inner membrane. J. Biol. Chem. 269:11306-11311.

BOOK CHAPTERS:

1.Moore, MAS, Han, W., and **Ye, Q.**, (2000). Notch signaling during hematopoietic development. In "Developmental Biology of Hematopoiesis" ed. Zon LI. Oxford Univ. Press

2. **Ye, Q.**, Barton, RM., and Worman, HJ. (1998). Nuclear Lamin-binding proteins. Subcell Biochem 31:587-610.

SELECTED ABSTRACTS AND PRESENTATIONS:

1. **Ye, Q.,** Wang, J., Robinson, B., Ragusa, D., Rousseva, V., Edinger, J, Nelson, R. Hofgartner, W., and Hariri, R. Evaluation of abbott Cell-Dyn 1700 and Cell-Dyn3200 automated hematology analyzers for enumeration of total nucleated cells isolated from human placenta. International Conferences on Stem Cells Research and Therapeutics April 11-13, 2005, San Diego, CA,

2. **Ye, Q.,** Wang, J., Edinger, J. Magidson, J. Rousseva, V., Maclsaac, S., Hofgartner, W., and Hariri, R. Isolation and differentiation of pluripotent stem cells from human placentas (Oral Presentation). International Conferences on Stem Cells Research and Therapeutics. April 11-13, 2005 San Diego, CA.

3. Wang, J., **Ye, Q.,** Edinger, J. Rousseva, V., Zeitlin, A., Hofgartner, W., and Hariri, R. In vitro study of co-culture of human placental perfusate and cord blood. International Conferences on Stem Cells Research and Therapeutics April 11-13, 2005 San Diego, CA.

4. **Ye, Q.,** Payvandi, F., Wu, L., Zhang, L., Muller, G., Chen, R., McClellan, S., Wang, J., Khorshidi, M., Magidson, J., Stengel, J., Stirling, D., and Hariri. R. (2002). *Novel IMID Drugs enhance expansion and regulate differentiation of human cord blood CD34+ cells with cytokines.* The American Society of Hematology 44th Annual Meeting. Philadelphia, PA. Dec.6-10. Blood. (2002) 100: Abstract No.4099

5. Wang, J., Struck, M., A., McClellan, S., **Ye, Q.**, Maclsaac, S., Lakato, T., Stengel, J., and Hariri, R. (2002) 2nd Annual Conference on Mesenchymal and nonhematopoietic stem cells. Sept. 26-28. New Orleans. LO. *Stem cells from placenta differentiate into neural cells in vitro.* Abstract No.49.

6. Wang, Y, **Ye, Q**, Cioffi J, Khorshidi, M., Magidson, J., Katz, R., Maclsaac, S., and Hariri, R.. *Enhanced recovery of hematopoietic progenitor and stem cells from cultivated, postpartum human placenta.* The American Society of Hematology 43rd Annual Meeting, Orlando, Florida, Dec 7-11, 2001. Blood (2001) 98: Abstract No.769

7. **Ye, Q.**, Wang, Y., Cioffi, J., Khorshidi, M., Magidson, J., Katz, R., Maclsaac, S., and Hariri, R. *Recovery of Placental-Derived Adherent Cells with Mesenchymal Stem Cell Characteristics.* Blood (2001) 98: The American Society of Hematology 43rd Annual Meeting Orlando, Florida, Dec 7-11. Abstract No. 4260

8. **Ye, Q.**, and Malcolm A.S. Moore (2000) *An activated Notch 4 (Int-3) affects the proliferation and differentiation of hematopoietic stem/progenitor cells.* The American Society of Hematology 42nd Annual Meeting, San Francisco, California, Dec.1-5. Abstract No.280

FACS SUMMARY

ID #	Medium	Frozen (Vials)	CD34	CD45	CD10	CD29	CD54	SH2	SH3	SH4	SSEA4	CD44	HLA1	CD90	(Oct4)
PLSC-1	BW	Yes (2)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos					
PLSC-2	BW	Yes (6)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos					
PLSC-3	BW	Yes (2)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos					Pos
PLSC-4	BW	None													
PLSC-5	BW	Yes (9)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos					
PLSC-6	BW	Yes (26)	Neg	Neg	Pos/Low	Pos	Pos	Pos	Pos	Pos					Pos
PLSC-7	BW	Yes (2)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos					Pos
PLSC-8	BW	Yes (10)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos					Pos
PLSC-9	BW	Yes (11)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos					Pos
PLSC-10	BW	Yes (10)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos					Pos
PLSC-11	DMEM-5%FCS	Yes (9)													
PLSC-12	DMEM-5%FCS	Yes (7)													
PLSC-13	DMEM-5%FCS	Yes (5)													
PLSC-14	DMDM-5%FCS	Yes (9)													
PLSC-15	Anthro-1	Yes (7)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos		Pos	Pos	Pos	
PLSC-16	Anthro-1	Yes (8)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos		Pos	Pos	Pos	
PLSC-17	Anthro-1	Yes (8)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos		Pos	Pos	Pos	
PLSC-18	Anthro-1	Yes (8)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos		Pos	Pos	Pos	
PLSC-19	BWtoAnthr-1	Yes (17)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos		Pos	Pos	Pos	
PLSC-20	BWtoAnthr-1	Yes (40)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos		Pos	Pos	Pos	
PLSC-21	BWtoAnthr-1	Yes (9)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Pos/Neg	Pos	Pos	Pos	
PLSC-22	BWtoAnthr-1	Fail to Expand													
PLSC-23	Anthro-1	Yes (10)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos		Pos	Pos	Pos	
PLSC-24	Anthro-1	Fail to Expand													
PLSC-25	Anthro-1	Fail to Expand													
PLSC-26	Anthro-1	Yes (15)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos		Pos	Pos	Pos	
PLSC-27	Anthro-1	Yes (25)	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos		Pos	Pos	Pos	

FIGURE 4

FACS ANALYSIS OF SH2+, SH3+, SH4+ PLACENTAL PERFUSATE CELLS

MARKER	YW2073	YW2059
CD34	–	–
CD45	–	–
CD10	+	+
CD29	+	+
CD44	+	+
SH2	+	+
SH3	+	+
SH4	+	+
SSEA-3	–	–
SSEA-4	–	–

FIGURE 6